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INJECTION EXPERIMENTS ON PLUM TREES IN RELATION TO *STEREUM PURPUREUM* AND SILVER-LEAF DISEASE

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Introduction

In connection with investigations on Silver-leaf disease Brooks and Moore (1) pointed out in 1926 that certain pathological symptoms developed in the leaves when stems of plum trees were injected with the culture fluid in which *Stereum purpureum* had been grown, but from which all traces of the living fungus had been removed. These symptoms involved a yellowish mottling or a wilting and browning of the leaves immediately above, or otherwise near, the places of injection. In those early experiments, carried out from 1923–25, the injections never resulted in silvering of the foliage. During the last three years many injection experiments, chiefly of a somewhat modified type, have been performed, and these have demonstrated conclusively that silvering of the foliage as well as other pathological symptoms can be induced by injecting plum trees with non-living extracts of *Stereum purpureum* in the used culture fluid or by injecting them alone with the culture fluid in which the fungus has been grown.

The mode of injection of the stems of these trees was that described by Brooks and Moore (1), which has been found entirely satisfactory. In the most recent experiments a fairly large reservoir has been attached to the intake tube in order to obviate the need for frequent replenishment of the injection fluid. During the last three years these experiments have been commenced in March, i.e. at the time when the buds of the plum trees (in an unheated greenhouse) are beginning to expand, in order to bring the developing leaves under the influence of the injection fluid throughout their growth. As the rate of intake of liquid at any one injection hole falls rapidly after about ten days, a series of injections were made at intervals, vertically above one another, so that the expanding buds in one particular region of the tree were constantly affected by the injection fluid. As the injection holes ceased to be used they were closed with sealing wax.

Preparation of fluids used for injection

As experience had shown that the most profuse mycelial growth was obtained by growing the fungus on sterilised plum twigs standing in an asparagin-glucose-starch-mineral salt medium this kind of culture was invariably used in the experiments. Growth of the fungus was started from spores on a solid medium in Petri dishes, and when it was certain that a pure culture had been obtained, small portions of mycelium were transferred to the upper extremities of the plum twigs in the culture flasks. The mycelium grew down the twigs into the nutrient fluid, on the surface of which a dense mycelial mat was formed. The reason for inoculating first the upper extremities of the twigs rather than the culture fluid direct was to obviate the risk of contamination. If mould fungi obtained access to the flasks during inoculation their colonies could be seen either on the twigs or in the fluid medium before the mycelium of *Stereum purpureum* reached the latter, and such flasks could be discarded. Without this precaution the purity of the cultures could not be guaranteed.

As silvering did not result in the 1923–25 experiments by using for injection the filtered fluid in which the fungus had grown, it was decided in later experiments to obtain an extract of the fungus for injection in the following manner: a portion of the mycelial mat together with some of the used culture fluid was removed from the flask, placed in a mortar containing sterilised sand, pounded up vigorously, and then filtered through ordinary filter paper into sterile tubes. The fluid thus obtained, diluted if required with sterile water, was used immediately for injection. Previous experience had shown that it was impossible to keep the injection fluids entirely free from bacterial contamination under the conditions obtaining in these experiments, so filtering through a Berkefeld bougie, as in the earlier experiments, was dispensed with. In using ordinary filter paper precautions had to be taken to use mycelium entirely free from the spores as the latter were found to pass through such paper. Spores of *S. purpureum* are rarely produced under the conditions of culture described above, and then only in very old cultures, so the risk of spores being present was entirely avoided by using young mycelium. The fluid obtained in this way for injection was quite clear and usually remained so for several days, but if it became cloudy owing to bacterial contamination it was discarded forthwith. Toluol was sometimes added to this kind of injection fluid in order to inhibit the development of bacteria, but, as will be pointed out later, this made no difference to the results obtained on injection.

The culture fluid in which the fungus had grown was also used for injection after filtration.

Portions of these fluids were boiled for five minutes before being used for injection, and control experiments were carried out, in which sterile distilled water, distilled water + toluol, and the culture medium, were used for injection.

RESULTS OF INJECTION EXPERIMENTS

(A) *With fluid obtained after pounding up young mycelium in the liquid medium in which it had grown*

Eight young Victoria plum trees were injected from about the end of March onwards. About 4–7 days after injection the tips of the sepals and petals of many flowers immediately above the injection hole became brown, and the browning often extended to the base of these organs. Shortly afterwards, the tips or margins of young leaves in the same region also became brown. These symptoms appeared somewhat later in higher parts of the trees, especially on the same side as the injection holes, and, to a limited extent and more erratically, below the lowest place of injection. At the top of these trees the affected leaves were irregularly distributed. A little later still, some of the leaves with brown tips developed a number of small, irregular yellow spots in the lamina, which fell away after a time, leaving holes. The brown tips or margins of the leaves also fell off. Subsequently, many of the leaves on each of the eight trees became silvered. On examination, these silvered leaves showed the same histological symptoms as those characteristic of silvered plum leaves in nature.

Two other Victoria plum trees were injected with this fluid to which a few drops of toluol had been added. The effects on the flowers and foliage were the same as when toluol was omitted.

Some of the fluid obtained after pounding up the mycelium was diluted with sterile water to one-quarter strength and was then used for injecting seven other Victoria plum trees. The results were identical with those obtained by injecting with the full-strength fluid. With two young Czar plum trees, however, injected with this diluted fluid, the only pathological effect was silvering of the leaves, there being no browning of the flowers or leaf-tips.

(B) *With fluid as in* (A), *but boiled for five minutes before injection*

Two young Victoria plum trees were injected with this fluid, but although browning of the flowers and leaf-tips occurred the leaves did not become silvered.

Two other Victoria trees injected with this fluid, diluted to one-quarter the normal strength, gave the same result. A Czar plum tree injected with the same diluted fluid showed no pathological symptoms at all.

(C) *With the fluid in which the young mycelium had been growing*

Of four young Victoria plum trees which were injected with this fluid all showed browning of the flowers and leaf-tips, but only three became silvered subsequently. A young Czar plum tree treated in the same way showed no browning of the flowers or leaf-tips, but became silvered. These results differ from those obtained with the same kind of fluid used in the 1923–25 injections described by Brooks and Moore (1), when there was no silvering of the foliage.

(D) *With fluid as in* (C), *but boiled for five minutes before injection*

One young Victoria plum tree, injected with this fluid, showed browning of the flowers and leaf-tips, but no silvering, and a Czar plum tree, similarly injected, exhibited no pathological symptoms of any kind.

(E) *With fluid obtained after pounding up old mycelium in the liquid medium in which it had grown*

The injection fluid was prepared in the same way as "fluid (A)," but the mycelium used for this purpose was two years old, the culture flasks having remained free from contamination. No sporophores had developed in these cultures, but, for the reasons stated earlier in this paper, there was no guarantee that spores were not present in the injection fluid, although this was unlikely.

Two young Czar plum trees were injected with this fluid diluted to one-quarter of the normal strength; both became silvered, but only one of these trees showed browning of any of the leaf-tips.

Another Czar plum tree, injected with some of this fluid which had been boiled for five minutes, showed no pathological symptoms.

(F) *With the fluid in which old mycelium had been growing*

This fluid was that in which the mycelium referred to in connection with "fluid (E)" had been growing for two years. Two young Czar plum trees were injected; both became silvered, and one showed browning of the tips of some small leaves before silvering was apparent.

(G) *Control experiments*

Other young Victoria and Czar plum trees were injected with the fresh culture fluid, with distilled water, and with distilled water + toluol, but these injections had no adverse effect upon the trees.

These results afford convincing proof that the same effect, viz. silvering of the foliage, can be produced in plum trees by injecting them with an extract of the mycelium in the used culture fluid, devoid of all traces of the living fungus, as by inoculation with *Stereum purpureum*. In the more recent experiments silvering has been induced also by injecting trees with the fluid in which the fungus has been growing for some time, hence the substance which directly or indirectly causes silvering is present in this fluid. Presumably, this substance passes out from the mycelium into the fluid. The minimum time between the commencement of injection and the onset of silvering was 15 days, a period considerably shorter than that which elapses between inoculation with the living fungus and the appearance of silvering of the foliage. The shortness of the period between injection and the development of silvering affords indirect proof that the pathological symptoms were not induced by chance inclusion of portions of the living fungus. It is to be expected that in these injection fluids there would be a greater concentration of the substance or substances which lead to silvering than would be produced during the early stages of development of mycelium in the tissues.

With Victoria plum trees the onset of silvering was preceded by browning of the flowers and leaf-tips as well as by other pathological symptoms occasionally. In this connection it is noteworthy that silvered Victoria trees, which contain the living fungus, sometimes show these symptoms also during the unfolding of the buds, although in the writers' experience these effects are never so striking as in the injected trees. With injected Czar plum trees, however, these additional pathological effects were apparent only on two occasions.

When the above fluids were boiled for five minutes before injection no silvering resulted, although with Victoria plum trees browning of the flowers and leaf-tips was still evident. It appears therefore that one of the substances associated with the causation of pathological symptoms in silvered trees is thermo-stable.

The fact that silvering was induced by injecting fluid in which the fungus had grown for two years indicates that the agent which causes silvering is probably stable for long periods at ordinary temperatures.

Many parasitic organisms are now known to secrete substances of a toxic nature which cause symptoms of disease in parts of the plant remote from the actual seat of the parasite. For instance, Hutchinson (2) demonstrated in 1913 that the wilting symptoms of tobacco plants attacked by *Bacterium solanacearum* are induced by a toxin secreted by this organism, and, more recently, Brandes (3), Bewley (4) and Dowson (5, 6) have shown that yellowing and wilting of the foliage of plants affected by species of Fusarium and Verticillium[1] are caused by the secretion of toxic substances, which are carried up to the leaves in the transpiration stream. The behaviour in this respect of *Stereum purpureum* in the tissues of woody plants is essentially the same, but the pathological symptoms induced by the secretion of toxic substances by this fungus appear to be more diverse in character than those caused by the other organisms mentioned.

Examination of the woody parts of some of the injected trees

Two of the trees injected with "fluid (A)" were cut up for examination four months after injection had begun, i.e. a considerable time after silvering had become apparent. The xylem on the side of the injection holes was found to be discoloured, owing to the accumulation of gum-like substances, just as in trees invaded by *Stereum purpureum*, the discoloration extending for several inches above and below the injection holes. The discoloration, however, did not pass into the small lateral branches or into the top of the tree, both of which bore silvered leaves. Sections of the discoloured wood showed hyphae occasionally; this is not surprising in view of the lapse of time since the injections had been started and of the impossibility of carrying out these experiments under completely aseptic conditions. The fungi occasionally found in the discoloured wood were isolated in culture in the usual way, but *S. purpureum* was never obtained. It was concluded therefore that "fluid (A)" was capable of producing the same brown discoloration of the wood as is caused by the living mycelium.

Two other trees injected with "fluid (B)" (i.e. "fluid (A)" which had been boiled for five minutes) were examined in the same way. Although these trees exhibited only browning of the flowers and leaf-tips the amount and distribution of the discoloured wood in the main stem was essentially the same as in the trees injected with

[1] The organism described by Dowson was referred to by him as a species of Cephalosporium, but it is now known to be a species of Verticillium.

"fluid (A)." It would appear therefore that though boiling had destroyed the agent which causes silvering of the foliage, the high temperature had not affected the capacity of the fluid to cause extensive gum-formation in the wood. The hyphae occasionally found in the discoloured wood of these trees were not those of *S. purpureum*.

With trees injected with water or with the fluid used for growing the fungus gum-formation occurred only in the immediate vicinity of the injection holes.

CONDITION OF THE TREES IN THE YEAR FOLLOWING INJECTION

All the trees which became silvered or showed other pathological symptoms when injected exhibited normal flowers and foliage in the following year. This is a further indication that the fluids used for injections were devoid of living *S. purpureum*, as in all probability some of these trees at any rate would have been silvered again in the following year if *S. purpureum* had been inadvertently introduced into them.

SUMMARY

(1) Silvering of the foliage and other pathological symptoms, such as browning of the flowers and leaf-tips, are induced in plum trees by injecting their stems with a filtered, non-living extract of *Stereum purpureum* in the culture fluid in which the fungus has grown.

(2) The same effects are produced by using for injection only the culture fluid in which the fungus has grown for some time.

(3) Boiling of these fluids for five minutes before using them for injection inhibits silvering of the foliage but does not prevent the development of other pathological symptoms.

(4) These fluids, whether boiled or unboiled before injection, induce an extensive brown discoloration of the wood in the vicinity of the injection holes owing to the formation of gum-like substances. This effect on the wood is similar to that caused by the growth of *Stereum purpureum* in it.

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